-53-

Again Minipreps for Sequencing

MINIPREP FOR DNA PREPARATION

Old: 1, 3, 4, 5, 6, 10,
New: 13, 15, 16, 17, 18, 19, 20

1] INOCULATE CLONES INTO 3.0 ML OF LB MEDIA OVERNIGHT AT 37 DEG.

2] THE FOLLOWING DAY LABEL 1.5 ML. STERILIZED EPPENDORF TUBES TO BE USED. 2 ml

3] FILL 3/4 OF EACH TUBE WITH THE CULTURED BACTERIA.CLOSE THE CAP

4] SPIN TUBES USING THE EPPENDORF CENTRIFUGE FOR ONE MINUTE AT 6000 RPM.
PREPARE THE POT FOR BOILING WATER

5] DECANT THE SUPERNATANT, LEAVING A LITTLE BIT IN THE TUBE.

6] RESUSPEND THE PELLET BY VORTEXING

7] ADD 120 micro ml OF STEL-T SOLUTION . MIX GENTLY BY INVERTING THE TUBES. DO NOT VORTEX. 240 µl

8] KEEP THE TUBES IN BOILING WATER FOR EXACTLY 1 MINUTE AND THEN IMMEDIATELY TRANSFER THE TUBES TO A BUCKET OF ICE.
After boiling the DNA appears whitish in colour.

9] KEEP THE TUBES IN ICE FOR 5 MINUTES. → 10'

10] SPIN THE TUBES IN THE MICROFUGE FOR 5 MINUTES AT THE HIGHEST SPEED. (12 x 1000 RPM)

11] USING A STERILIZED TOOTHPICK PICK UP THE PRECIPITATE AND DISCARD IT, LEAVING THE SUPERNATANT BEHIND.

12] ADD 120 micro ml OF 2-PROPRANOL (same quantity as the STEL-T solution) 2-ISOPRANOL PRECIPITATES THE DNA. MIX THE TUBES BY INVERSION AND KEEP ON ICE FOR 5 MINUTES. 240 µl

13] SPIN FOR 5 MINUTES IN THE MICROFUGE AT THE HIGHEST SPEED. (12x1000 RPM)

14] DECANT THE SUPERNATANT AND LEAVE THE TUBES TO AIR DRY IN AN INVERTED POSITION.
(To hasten this step, you may add 200 micro ml of 100% alcohol without disturbing the pellet and allow to air dry as before.)
→ discard the alcohol.

15]ONCE THE PELLET IS DRY, WHICH CAN BE MADE OUT BY THE POWDERY APPEARANCE OF THE PELLET, RESUSPEND IN 60 micro ml OF TE WITH RNAase. 120 µl

WORKING SOLUTION OF TE WITH RNAase

2 micro ml of RNAase (20 mg/ml) in 1 ml of TE

16]THESE DNA CAN BE FROZEN AT -20 TEMPERATURE IF REQUIRED.

Digest: • 25 µl DNA
3.2 µl 10x Buffer 3
3 µl 10x BSA
0.5 µl NotI
0.5 µl BglII

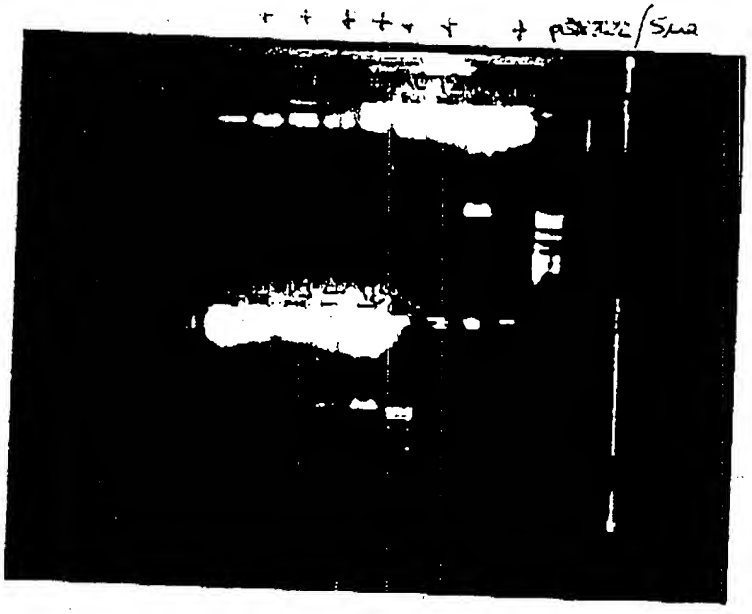
x14 → 44.8 µl
42 µl
7 µl
7 µl
} Take 7 µl to DNA

• 1 µl BSA + 24 µl H2O

2h 37°C.

Rat 100µl → ad 1000µl TC
→ 200µl PCIA → 6
→ 200µl CIN → ⊗ is gone
→ 2ml NaCAc
→ 50µl EtOH 1h -20°C
→ 1x 70% EtOH
↓
Sequencing

Rat-Bill digest:



⊗ Sequencing 3, 4, ~~5~~, 10, 13, 16

Sequencing:

5' primer Gussens TBOUMO → Schwedenen

3' primer CT-BKL-STG primer

Clones 3, 4, 10, 13, 16

kit: USB → Rockville "Benchmarks"

→ Resuspending in 2 µl H_2O.

1 µl DNA
2 µl 5x Sequenase
1 µl 5% Nonidet.
6 µl H_2O

} per Bertrand 3' end

↓

100°C 3-4 min.

↓

dry ice (15') perhaps longer

↓

15 sec spin

↓

Ice.

Re Reaction: 1 µl S-ATP (7000 Ci/mmol)
1 µl DTT kit [100 mM]
2 µl Labelling Mix & prediluted 1:5
2 µl Sequenase 1:8 diluted.

} premix, use
10 µl
7 µl
2 µl
→ 6 µl / reaction

↓

4 min RT.

3,5µl → to 2.5µl aliquoted ddNTP
↓
5' 37°C.
⊕ 4µl Stop solution.

→ 5% Sequencing Gel: 4.75g PAA
0.25g Bis
42g Urea
10ml 10xTBE
500µl APS 10%
40µl Temed.

Loading:
5' Reaction 3, 4, 10, 13, 16
3' Reaction 3, 4, 10, 13, 16.

Run: 1322V /34mA /45Watt

Exposure 4⁰⁰ – 11⁰⁰ → 5h.

Animpfen von Bacterien: 1, 3, 4, 5, 6, 10, 13, 16, 17, 18, 19

Sequence

=> 5' Reaction couldn't work, because TBG-VCHO is priming in CD8 α-leader!

=> 3' Reaction:

CCC GNCNAGA | GCG | ATA | GGC | CGC | GAA | CTC GGT GGT
GGG CNG TCT | CGC | TAT | CCG | AACG | CTT | GAG CCA CCA

3) GCA TAG GGC TGC TAA TGC TAG CGG GTA GGG
CGT ATC CCG ACG ATT ACG AAC GCC CAT CCC

GGNCGGCGGG AGT | CAT | GTT | CAT | GTA |
TCA | GTA | CAA | GTA | CAT |

GTC | ACT | GTG | CAG | GAG | CCT | GCT | CTT | ACT | CTC | A CC
CAG | TGA | CAC | GTC | CTC | GGA | CGA | GAA | TGA |

GAA ATA A ACT A

4) GTC AGA | G | ATA | GG | | GAA | GAC | GCG | TGG

ATG | CTT |

AGT | CAT | GTA | CAT | GTA

ACT | CAT G |

14) GTC ATA

TGG | GGC |

AGT

GGA CAA GAA TGA

13)

16)

Results, all have CD28-Insert → look 5' Prime!